View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector

RAT LIVER PROTEINS BINDING AND TRANSFERRING PHOSPHATIDYLSERINE

Jolanta BARAŃSKA and Zenon GRABAREK

Nencki Institute of Experimental Biology, Departments of Cellular Biochemistry and Biochemistry of Nervous System and Muscle, Pasteura 3, 02–093 Warszawa, Poland

Received 12 June 1979

1. Introduction

Transfer of phospholipids between cellular membranes is mediated by a class of transfer proteins present in the cytoplasm of several tissues. Proteins transferring phosphatidylcholine and phosphatidylinositol have been identified, characterized and purified [1,2]. More recently, two fractions of low molecular weight proteins (around 13 000) capable of transferring phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol, sphingomyeline and cholesterol were also isolated from rat liver [3]. The amino acid composition of one of these proteins was already described [4].

The transfer of phosphatidylserine mediated by a soluble fraction of rat liver homogenate was first described by Butler and Thompson [5]. More recently, Zilversmit and Hughes [6] presented evidence that a partially purified protein fraction of rat liver can transport phosphatidylserine from microsomes to mitochondria.

The present study provides more information on the transfer proteins for phosphatidylserine. It demonstrates the ability of these proteins to bind phosphatidylserine and a high sensitivity of this binding to the ionic strength. Amino acid composition of two low molecular weight transfer proteins is also described.

2. Materials and methods

Liver mitochondria and microsomes from male Wistar rats were isolated by a conventional procedure. Labeled microsomes were made by incubating microsomal suspension with [^{14}C]serine, [^{14}C]ethanolamine or [^{14}C]choline as described previously [7]. Liposomes made of total microsomal phospholipids containing [^{14}C]phosphatidylserine, [^{14}C]phosphatidylethanolamine or [^{14}C]phosphatidylcholine were prepared as described [7]. Partially purified transfer proteins were obtained essentially according to Bloj and Zilversmit [3]. The procedure included: precipitation of the 100 000 × *g* supernatant of liver homogenate at pH 5.1, ammonium sulphate cut between 50% and 95% saturation, gel filtration through Sephadex G-75 in 10 mM Tris–HCl/5 mM mercaptoethanol/1 mM NaN_3 (pH 7.4), chromatography on CM-52 cellulose equilibrated with 10 mM Tris–HCl/1 mM mercaptoethanol (pH 7.9) and eluted with 50 mM Tris–HCl/1 mM mercaptoethanol (pH 7.9), heating the pooled fractions containing transfer activity for 5 min at 90°C and removing denatured proteins by centrifugation. Phosphatidylserine, phosphatidylethanolamine and phosphatidylcholine transfer activities were determined by incubation of the labeled liposomes with mitochondria and the exchange protein. The transfer activity was expressed as the decrease of radioactivity in the supernatant after sedimenting mitochondria, in percentage of the control without exchange protein.

Electrophoresis was performed on 15% polyacrylamide slabs in the presence of SDS according to Weber and Osborn [8]. For the amino acid analysis the protein components were recovered after preparative electrophoresis by extraction with 0.1% SDS followed by concentrating and desalting on Sephadex G-25. Analysis was performed after hydrolysis with 6 N HCl for 20 or 70 h at 110°C with Beckman 119CL amino acid analyzer according to

Spackman et al. [9]. Cysteine content was estimated as cysteic acid after performic acid oxidation [10].

3. Results

When cytoplasmic proteins of rat liver were applied on a column of Sephadex G-75, the ability to transfer phosphatidylserine was resolved into two peaks (fig.1). Two similar peaks were also found for the transfer of phosphatidylethanolamine. These results were confirmed by the use of [^{3}H]glycerol trioleate tracer in liposomes which served as a nonexchangeable reference marker. The first peak (fractions no. 4–9 in fig.1) coincided with the void volume of the column and therefore corresponded to high molecular weight proteins, the second one (fractions no. 25–31 in fig.1) contained low molecular weight proteins and also possessed phosphatidylcholine transfer activity (not shown).

Both high and low molecular weight protein fractions could bind [^{14}C]phosphatidylserine when incubated with labeled microsomes. When the high molecular weight fraction containing labeled

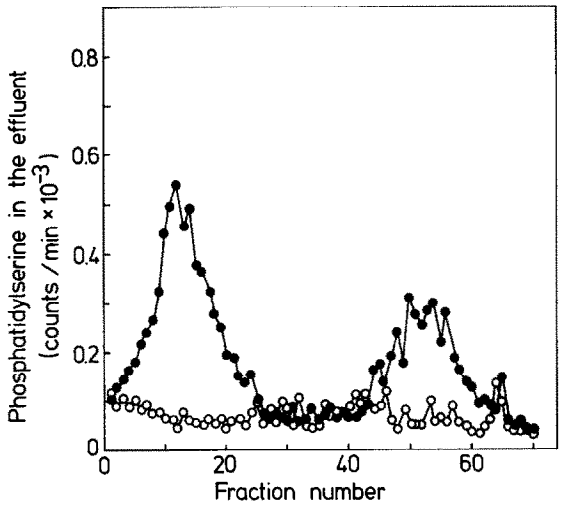

Fig.2. Elution profile of the high molecular weight fraction loaded with [^{14}C]phosphatidylserine. 42 mg of high molecular weight proteins (fractions no. 4–10 of fig.1) were incubated with microsomes (1.3 μmol total phospholipid phosphorus) containing [^{14}C]phosphatidylserine (215 000 cpm) in 5 ml of 10 mM Tris–HCl/5 mM mercaptoethanol/1 mM NaN_3 (pH 7.4) for 1 h at 37°C and re-filtered through Sephadex G-75. 2 min fractions were collected (•). As the control, labeled microsomes were incubated with the elution medium alone (○).

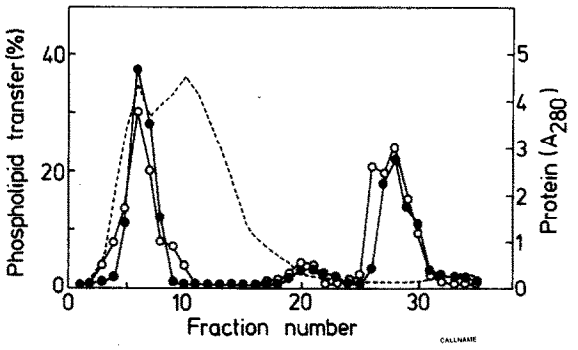

Fig.1. Gel filtration of transfer proteins for phosphatidylserine and phosphatidylethanolamine of rat liver cytoplasm. The proteins obtained by ammonium sulphate precipitation were filtered through Sephadex G-75. The flow rate was 60 ml/h, and 4 min fractions were collected. Aliquots of each fraction were incubated for 2 h at 37°C with liposomes (0.17–0.26 μmol total phospholipid phosphorus) containing [^{14}C]phosphatidylserine (25 000 cpm) or [^{14}C]phosphatidylethanolamine (25 000 cpm) and mitochondria (6 mg protein) in 1.2 ml of 50 mM sucrose/10 mM Tris–HCl/1 mM EDTA (pH 7.4). (•————•) Transfer of phosphatidylserine; (○————○) transfer of phosphatidylethanolamine; (– – – –) protein content.

phosphatidylserine was again filtered through Sephadex G-75, the label was eluted in fractions corresponding to both high and low molecular weight proteins (fig.2). In contrast, the low molecular weight fraction loaded with [^{14}C]phosphatidylserine showed, after re-filtration, only one peak of radioactivity in the low molecular weight region. Thin-layer chromatography of lipids extracted from this fraction demonstrated the presence of [^{14}C]phosphatidylserine. It was also found that the binding of [^{14}C]phosphatidylserine from microsomes to the low molecular weight proteins was strongly decreased with increasing ionic strength of the medium (fig.3).

Chromatography on CM-cellulose resulted in a further purification of the transfer activity for phosphatidylserine, amounting to 412-fold and 303-fold for the low and the high molecular weight fractions, respectively, as compared with the initial pH 5.1 supernatant. The active proteins of both fractions were also able to transfer phosphatidylcholine to a similar extent as phosphatidylserine. Heat treat-

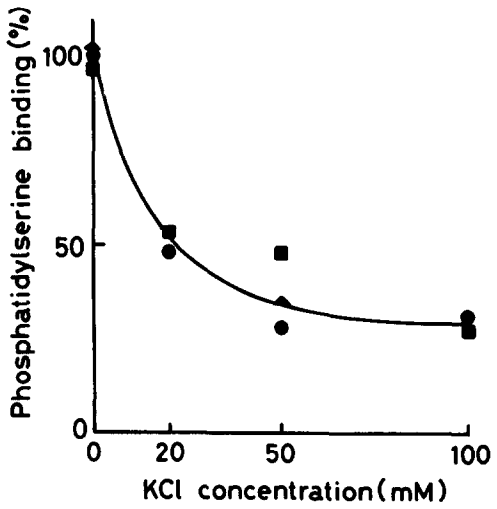

Fig.3. Effect of KCl on [^{14}C]phosphatidylserine binding to low molecular weight proteins. The low molecular weight fraction after Sephadex G-75 filtration (1.5 mg protein) was incubated for 15 min at 37°C in 10 mM Tris–HCl/5 mM mercaptoethanol/1 mM NaN_3 (pH 7.4) with different amounts of KCl as indicated. Thereafter, microsomes (3 μmol total phospholipid phosphorus) containing [^{14}C]phosphatidylserine (400 000 cpm) were added and the incubation was continued for 1 h. The final volume was 2.5 ml. After incubation, microsomes were removed by centrifugation, the lipids were extracted from the supernatants and counted for radioactivity. The radioactivity in samples without KCl was taken as 100%. The symbols represent results of 3 experiments.

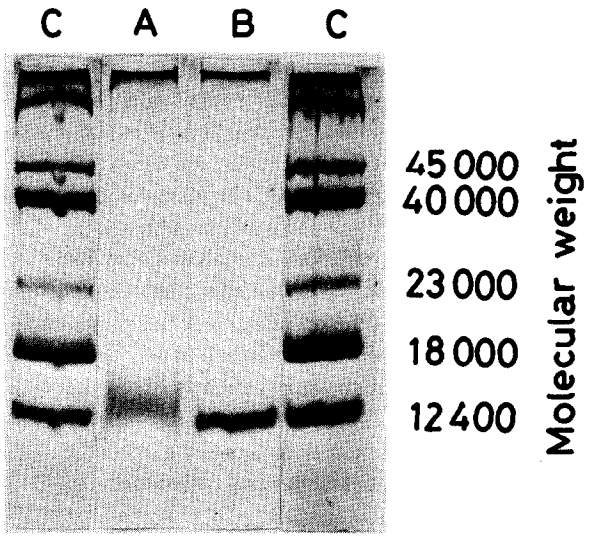

Fig.4. SDS–polyacrylamide gel electrophoresis phosphatidylserine transfer proteins. Slabs A and B show major proteins of the original high and low molecular weight fractions, respectively. Slabs C contain the following standards: cytochrome *c* (12 400), troponin C (18 000), troponin I (23 000), troponin T (40 000) and actin (45 000).

ment increased the purification factor to 539 and 394 for the low and the high molecular weight fractions, respectively. This procedure removed all high molecular weight proteins, so that SDS electrophoresis visualized only low molecular weight proteins in both fractions. The main component in both fractions had molecular weight of about 13 000. Moreover, minor protein impurities of 10 600, 16 500 and 21 000 daltons were found in the low and 16 600 and 21 000 daltons in the original high molecular weight fraction. The major protein bands of both fractions isolated from slabs and subjected again to SDS electrophoresis exhibited slightly different mobilities corresponding to molecular weights of 12 400 (comigrating with cytochrome *c*) and 13 200 for the low and the original high molecular weight fraction, respectively (fig.4).

Table 1 shows the amino acid composition of the

Table 1
Amino acid composition of two phosphatidylserine transfer proteins. The proteins of 12 400 and 13 200 daltons correspond to bands B and A of fig.4, respectively

Amino acid	12 400 dalton protein (mole %)	13 200 dalton protein (mole %)
Lysine	11.3	7.5
Histidine	2.2	4.3
Arginine	2.7	3.0
Tryptophan[a]	absent	present
Aspartic acid and asparagine	8.8	10.5
Threonine	8.5	4.7
Serine	5.5	7.1
Glutamic acid and glutamine	11.7	9.7
Proline	2.6	3.6
Glycine	9.8	13.5
Alanine	4.3	9.7
Half cysteine	1.4	2.6
Valine	9.1	6.0
Methionine	2.8	0.9
Isoleucine	6.1	3.1
Leucine	5.4	7.7
Tyrosine	2.7	2.3
Phenylalanine	4.8	3.9

[a] Concluded from the fluorescence spectrum

major proteins of both fractions. That of the low molecular weight fraction is characterized by a high proportion of lysine, the lack of tryptophan and a low content of cysteine, which is similar to the amino acid composition of the low molecular weight transfer protein already described [4]. The latter protein lacks tyrosine, histidine and arginine which were detected in our preparation, though in a very low amounts. These differences, however, may result from experimental error due to the fact that our preparation was eluted from the polyacrylamide slabs. The amino acid composition of the major protein of the second fraction exhibited some differences as compared to the first one. They mainly concerned a different proportion of lysine, alanine and threonine.

4. Discussion

The transfer proteins of the low molecular weight fraction found in the present study, characterized by a thermal stability and low phospholipid specificity, are probably identical with the low specificity transfer proteins of the CM_2 fraction described by Bloj and Zilversmit [3]. The major protein of the low molecular weight fraction reported in the present study and that of CM_2 fraction purified to electrophoretic homogeneity [4] have molecular weight of 12 400 and contain a similar amino acid composition.

An interesting feature of the phosphatidylserine transfer protein of the low molecular weight fraction, described in this report, is its high sensitivity to ionic strength. It is in a sharp contrast with a low sensitivity to salt concentration of the phosphatidylcholine transfer protein [11,12]. It can be therefore speculated that phosphatidylserine is bound to the transfer protein mostly by ionic binding. A similar binding between negatively charged head groups of phospholipids and positively charged amino acid residues of proteins has been postulated for plasma lipoproteins [13] and the glycophorin–phosphatidylserine complex [14]. In contrast to this, the specific phosphatidylcholine exchange protein mostly binds the phospholipid due to hydrophobic interactions [15].

The next fraction capable of transferring phosphatidylserine, presented in this paper, was eluted in the void volume of the Sephadex column. However, a partial recovery of the phosphatidylserine–protein complex in the low molecular weight region, after re-filtration of the high molecular weight fraction loaded with phosphatidylserine (fig.3), indicates that it probably represents an aggregate of a low molecular weight transfer protein with large molecules. The heat stability and lack of specificity of this fraction is similar to that of the low molecular weight fraction. However, the major protein bands have a slightly different electrophoretic mobility in both fractions. Also, the amino acid analysis exhibits some differences between these proteins and points out that in rat liver cytoplasm at least two different low molecular weight proteins capable of transferring phosphatidylserine are present.

In a previous paper [7] we expressed some doubt whether the cytoplasmic fraction stimulates the transfer of phosphatidylserine or rather its decarboxylation by mitochondria. The present investigation makes it clear that the liver cytoplasm indeed contains proteins capable of transferring phosphatidylserine. However, this does not exclude the possibility that these proteins also possess the ability to promote the decarboxylation of phosphatidylserine by mitochondria.

Acknowledgements

The authors wish to thank Professor L. Wojtczak for his constant interest in this study and helpful discussions during preparation of the manuscript. Skillful technical assistance of Mrs Jadwiga Klonowska is gratefully acknowledged.

References

[1] Wirtz, K. W. A. (1974) Biochim. Biophys. Acta 344, 95–117.
[2] Zilversmit, D. B. and Hughes, M. E. (1976) Methods Membr. Biol. 7, 211–259.
[3] Bloj, B. and Zilversmit, D. B. (1977) J. Biol. Chem. 252, 1613–1619.
[4] Bloj, B., Hughes, M. E., Wilson, D. B. and Zilversmit, D. B. (1978) FEBS Lett. 96, 87–89.
[5] Butler, M. M. and Thompson, W. (1975) Biochim. Biophys. Acta 388, 52–57.
[6] Zilversmit, D. B. and Hughes, M. E. (1977) Biochim. Biophys. Acta 469, 99–110.

[7] Barańska, J. and Wojtczak, L. (1976) FEBS Lett. 71, 83–86.
[8] Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406–4412.
[9] Spackman, D. H., Stein, W. H. and Moore, S. (1958) Anal. Chem. 30, 1190–1206.
[10] Moore, S. (1963) J. Biol. Chem. 238, 235–237.
[11] Wirtz, K. W. A., Geurts van Kessel, W. S. M., Kamp, H. H. and Demel, R. A. (1976) Eur. J. Biochem. 61, 515–523.
[12] Johnson, L. W. and Zilversmit, D. B. (1975) Biochim. Biophys. Acta 375, 165–175.
[13] Jackson, R. L., Morriset, J. D. and Gotto, A. M., jr (1975) Molec. Cell. Biochem. 6, 43–50.
[14] Van Zoelen, E. J. J., Zwaal, R. F. A., Reuvers, F. A. M., Demel, R. A. and Van Deenen, L. L. M. (1977) Biochim. Biophys. Acta 464, 482–492.
[15] Kamp, H. H., Sprengers, E. D., Westerman, J., Wirtz, K. W. A. and Van Deenen, L. L. M. (1975) Biochim. Biophys. Acta 398, 415–423.